Development of novel derivatives of stilbene as potent macrocyclic compounds microtubule factors

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ABSTRACT

Microtubules (composed of α- and β-tubulin heterodimers) ubiquitous cellular polymers are important components of the cytoskeleton and play diverse roles within the cell, such as maintenance of cell structure, protein trafficking or chromosomal segregation during cell division. The polymers of tubulin play a pivotal role in mitosis and are regarded as an excellent target for chemotherapeutic agents to treat cancer. This review presents a brief overview of the synthesis and mechanism of action of new compounds targeting the dynamic of microtubule – tubulin polymerization/depolymerization. It is divided into the following parts: section I concerns targeting microtubules- tubulin-binding drugs derivatives of stilbene. In section II there are presented photoswitchable inhibitors of microtubule dynamics. Section III concerns using macrocyclic compounds as tubulin inhibitors. In this review, the authors focused primarily on reports produced inthe last five years and the latest strategies in this field.

1. Introduction

Microtubules ubiquitous cellular polymers are important components of the cytoskeleton and play diverse roles within a cell such as maintenance of cell structure, protein trafficking or chromosomal segregation during mitosis [1,2]. These are cylindrical polymers composed of α- and β-tubulin heterodimers (subunits). The dimensions of a heterodimer are: 4 nm x 5 nm x 8 nm and its molecular weight is about 100 kDa. These subunits associate longitudinally to form protofilaments and the protofilaments associate laterally to generate a microtubule. As a result of the association, most often of 13 protofilaments, a microtubule forms hollow tubes (hollow rods) whose length reaches several micrometres [3]. The result of this type of polymerization is that the two ends differ in their kinetics of attachment. At the so-called "plus" end, polymerization and depolymerization are faster than the "minus" end [4]. The ends of microtubules undergo rapid lengthening and shortening by the addition and removal of tubulin heterodimers, a phenomenon termed ‘dynamic instability’ (Scheme 1). Dynamic instability results from the hydrolysis of GTP bound to β-tubulin during or shortly after polymerization, which reduces its binding affinity for adjacent molecules. Growth of microtubules continues as long as there is a high concentration of tubulin bound to GTP. New GTP-bound tubulin molecules are then added more rapidly than GTP is hydrolyzed, so a GTP cap is retained at the growing end. However, if GTP is hydrolyzed more rapidly than new subunits are added, the presence of GDP-bound tubulin at the end of the microtubule leads to disassembly and shrinkage. Microtubules play a pivotal role in cell division and are regarded as an excellent target for chemotherapeutic agents to treat cancer.

The compounds that target microtubules and disrupt the normal function of the mitotic spindle have proven to be one of the best class of cancer chemotherapeutic drugs available in clinics to date. This group includes tubulin-binding agents, containing the *Vinca* alkaloids (*e.g.*, vinblastine (**1**) and vincristine (**2**), used to treat breast cancer, lymphomas, sarcomas or vinorelbine (**3**) administered in breast, lung cancer, sarcomas) interfering with microtubule structure as destabilizers, and the taxanes (*e.g.*, paclitaxel (**4**) and docetaxel (**5**) treating ovarian, breast cancer, lung cancer and many others) which promote microtubule stabilization preventing their disassembly (Fig. 1). Because these compounds have been widely discussed in the literature [5–9] we focused primarily on the last five years of research and the latest strategies in this field [10–14]. We concentrated on novel derivatives as potent of microtubule-targeting agents (MTAs), on azo compounds and investigation of photopharmacology and macrocyclic compounds and their

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Scheme 1. a) Structure and b) dynamic instability of microtubules.

Fig. 1. The chemotherapeutic drugs: vinblastine (**1**), vincristine (**2**), vinorelbine (**3**), paclitaxel (**4**) and docetaxel (**5**).

activity as MTAs.

2. Targeting microtubules-tubulin-binding drugs derivatives of stilbene

Microtubule-targeting agents, constitute a various group of molecules that bind to microtubules and affect their properties. Two general classifications of substances interacting with microtubules are distinguished. One of them was created based on the binding site of a given compound to a specific region of a heterodimer. The tubulin- binding sites for microtubule targeting agents are: taxanes site, laulimalide /peloruside site and *vinca* alkaloids site, colchicine site, maytansine site and pironetin site [9]. Considering the mechanism of action, there are two major types of microtubule targeting agents; a) microtubule stabilizers (stabilizing binders - “microtubule-stabilizing” - increasing the microtubular mass)) - compounds bonding to sites of taxanes and of laulimalide/peloruside, and b) microtubule destabilizers (destabilizing binders)- compounds bonding to sites of *Vinca* alkaloids, colchicine, maytansine and pironetin [15]. It should be noted that both types of MTAs inhibit the dynamic instability of tubulin polymerization. There are some marked tubulin inhibitors - paclitaxel/taxol (**4**) [16], vinblastine (**1**) [17] and vincristine (**2**) [18]. They interact with tubulin with the taxane or vinca

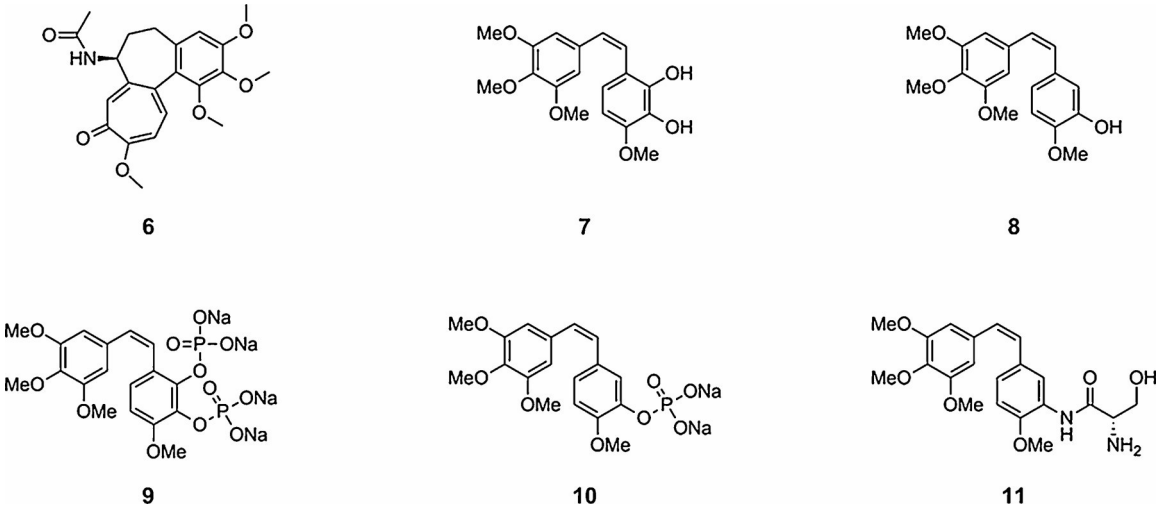


Fig. 2. The tubulin inhibitors targeting the colchicine binding site: colchicine (**6**), combretastatin A-1 (**7**), combretastatin A-4 (**8**), Oxi4503 (**9**), Fosbretabulin (**10**) and Ombrabulin (**11**).

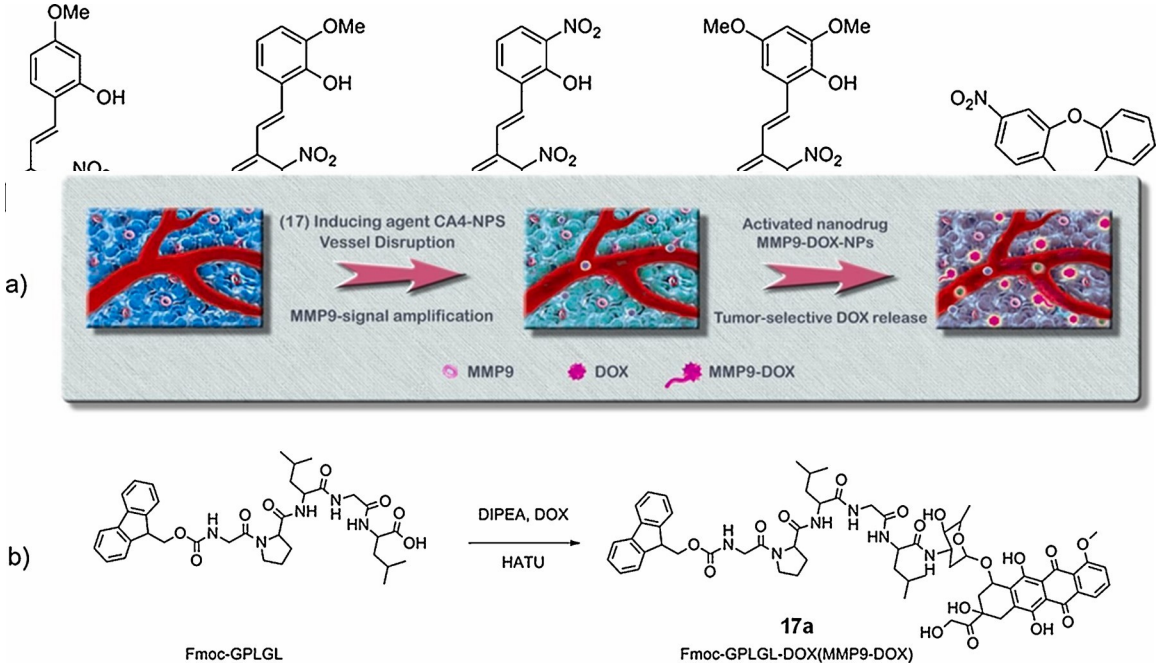


Fig. 4. a) Connecting combretastatin A4 nanodrug with MMP9-activated doxorubicin prodrug MMP9-DOX-NPs - nanodrug CA4-NPs (PLG-CA4, **17**); b) Synthesis of Fmoc-GPLGL-DOX (MMP9-DOX, **17a**) (according to [35]).

alkaloid binding sites. However, currently there have not yet been approved drugs of tubulin inhibitors targeting the colchicine (**6**) binding site (Fig. 2). Such compounds e.g. combretastatins analogs (Fig. 2): combretastatin A-1(CA1, **7**) and combretastatin A-4 (**8**) and their three prodrugs of monosodium phosphate salt two CA-1 P (Oxi4503, **9**) [19] and CA4P (Fosbretabulin, **10**) [20] or amino derivative AVE8062 (Ombrabulin, **11**) [21] have been extensively studied [19–22]. They can be transformed into the active components CA1 and CA4 *in vivo* [23], and have therapeutic advantages over taxanes and vinca alkaloids. They can be administered orally, have less

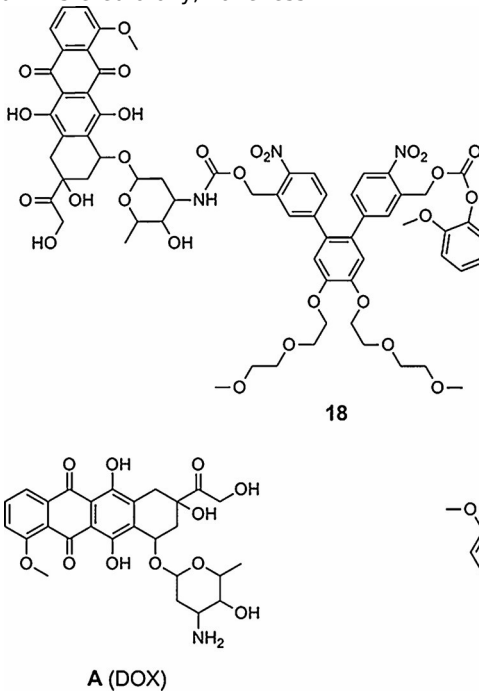


Fig. 5. The structure of photoresponsive hybrid prodrug (**18**) with which DOX part **A** and CA4 part **B** were covalently bonded.

drug-drug interaction potential, and are less prone to develop multi-drug resistance [6]. Some studies have been suspended due to lack of effects [24] or withdrawn due to the expected inability to recruit study participants in a reasonable amount of time [25] or due to limited funding [26]. Currently, only one research of OXi4503 is included in clinical trials - in phase 1. It focuses on the maximum tolerated dose of OXi4503 as a single agent and in combination with intermediate-dose cytarabine in subjects with relapsed/refractory Acute Myelogenous Leukemia or Myelodysplastic Syndromes [27].

Krawczyk et al. conducted a study concerning the synthesis and search for biologically active stilbene [28] and dibenzo[*b, f*]oxepin [29] derivatives. Dibenzo[*b,f*]oxepines have in their skeleton a (Z)-stilbene motif (like combretastatins). Moreover, dibenzo[*b,f*]oxepine is an important scaffold in medicinal chemistry. Its derivatives exhibit *anti*-depressive [30], antipsychotic [31], *anti*-estrogenic [32], antitumor [33] and *anti*-inflammatory [34] properties. The authors examined substituted stilbenes and discovered that compound (**12**) (Fig. 3) is effective against glioblastoma U87 cells with IC_{50} of 48.93 μM after 48 h.

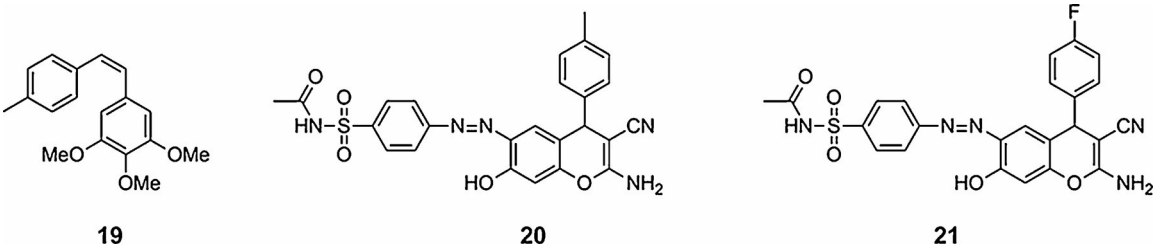
For active compounds, IC_{50} values were the lowest after 48 h of Cervical cancer HeLa treatment for four stilbenes (**12-15**) and one dibenzo[*b,f*]oxepin (**16**) and amounted 3.50, 17.18, 6.97, 17.05 and 18.54 μM, respectively. It is currently unclear if these compounds bind tubulin, because only molecular docking studies were performed. The interaction of (**12**) - (**16**) at colchicine binding site can be observed. There are also promising studies on different forms of combretastatins e. g. nanodrug (CA4-NPs, PLG-CA4, **17**, Fig. 4) [35]. This compound was built of a poly (L-glutamic acid) and combretastatin-A4 and its therapeutic effect was examined and compared with commercial combretastatin-A4 phosphate (CA4P) in a murine colon C26 tumor. PLG-CA4 showed significantly prolonged retention in plasma and tumor tissue.

The authors observed that the PLG-CA4 was mainly distributed around the tumor vessels and that its tissue penetration in solid tumors was low. Tumor suppression tests indicated that PLG-CA4 treatment resulted in a tumor suppression rate of 74 % and the rate of the CA4P group was 24 %. The authors also developed methods for the sequential delivery of CA4-NPs and MMP9-DOX-NPs (**17a**, matrix metalloproteinase 9 (typical tumor-associated enzyme)- activated doxorubicin prodrug, Fig. 4a) to enhance tumor-selective drug release in cancer therapy and which exhibits enhanced antitumor efficacy with reduced systemic toxicity compared with the noncooperative controls [36]. The MMP9-activated doxorubicin prodrug (Fmoc-GPLGLDOX, MMP9-DOX) was synthesized by conjugation of doxorubicin to Fmoc-GPLGL (Fig. 4b). MMP9-DOX was then encapsulated in methoxy poly (ethylene glycol)-b-poly(l-glutamic acid-co-L-phenylalanine) nanoparticles to yield MMP9-DOX-NPs. It was demonstrated that the combination of CA4-Nps MMP9-DOX-NPs exhibited superior anticancer efficacy compared with monotherapy. Recent studies of combretastatins focus on the combination of photoresponsive hybrid prodrug (**18**) that contains both doxorubicin (DOX, **part A**, Fig. 5) and combretastatin A4 (CA4, **part B**, Fig. 5) [37]. This is in order to apply photoremovable protecting groups (PPGs) in the field of combination chemotherapy.

When a sequential light stimulation strategy was applied for hybrid by 405 nm light for DOX and by 365 nm light for CA4 there was observed triggered drug release. Prodrug (**18**) improved exhibited significant

cytotoxicity (cell viability assay) for MDA-MB− 231 cells (human breast adenocarcinoma) compared with DOX and CA4 alone treated groups in both irradiated and nonirradiated conditions.

The search for tubulin inhibitors has also been conducted with derivatives of resveratrol, which is one of the most widely studied bioactive plant polyphenols having *anti*-cancer properties. The authors [38] showed that the derivative of resveratrol (**19**) (Fig. 6), induced cytotoxicity in several cancer cell lines *ex vivo* with an IC_{50} value of 3–5 μM. Investigation of the effect of (**19**) in non-small cell lung cancer cell line (A549) and T-cell leukemic cell line (CEM) demonstrated that it inhibited tubulin polymerization during cell division causing cell cycle arrest at G2/M phase of the cell cycle in 12–18 h period. Besides, immunofluorescence studies proved mitosis arrest after treatment (**19**) and indicated that this molecule binds to tubulin with a dissociation constant of 0.414 ± 0.11 μM. In turn, docking studies confirmed that (**19**) interacts with tubulin in colchicine binding site. Afifi et al. [39] presented a report regarding the development of a novel and primary model of synthesized sulfonamide compounds encompassing a chromene azo motif with the intent of becoming applicable for drug candidates in the cases of drug-resistant pathogens. The obtained molecules (**20**) and (**21**) (Fig. 6) were evaluated for their antibacterial and antifungal activities and their cytotoxic screening was performed against three cancer cell lines: human colon cancer cell line, human liver cancer cell line and breast cancer cell line. The incorporation of the chromene moieties into the azosulfonamide molecules improved cytotoxicity against their corresponding precursors. Some of these 4H-chromene/sulfonamide derivatives displayed potent antiproliferative activities, because they were more effective towards the examined tumor cell lines in comparison with the doxorubicin reference drug. Authors also studied the influence of two chosen derivatives (**20**) and (**21**) (Fig. 6) on the tubulin polymerization, using the HCT-116 cancer cell line. The results indicated the effect on the inhibition of the tubulin polymerization in comparison to colchicine (compound **20** 7.18 μM, compound **21** 8.84 μM, colchicine 0.16 μM). As we can observe from the data, the derivative (**20**) with an electron-donating substituent in phenyl ring (4− CH_3) demonstrated more activity in the selective comparison with (**21**) with an electronwithdrawing group in phenyl ring (4-F) for the inhibition of the tubulin polymerization.



19 20 21

Fig. 6. The structures of derivative of resveratrol (**19**) and azosulfonamide molecules (**20**) and (**21**) (destabilizing binders- compounds bonding to sites of colchicine).

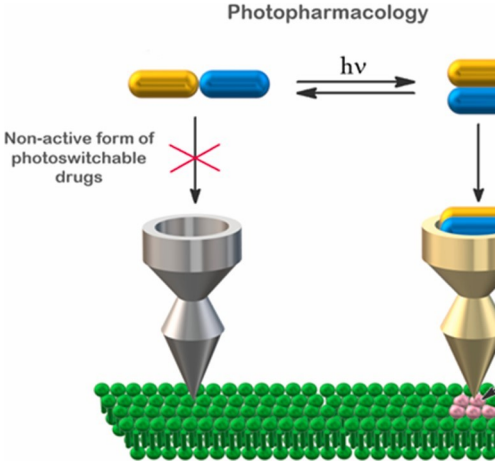


Fig. 7. The idea of the light-controlled photopharmacology- photoswitchable drugs that can be reversibly activated with light for interaction with their target receptors or enzymes.

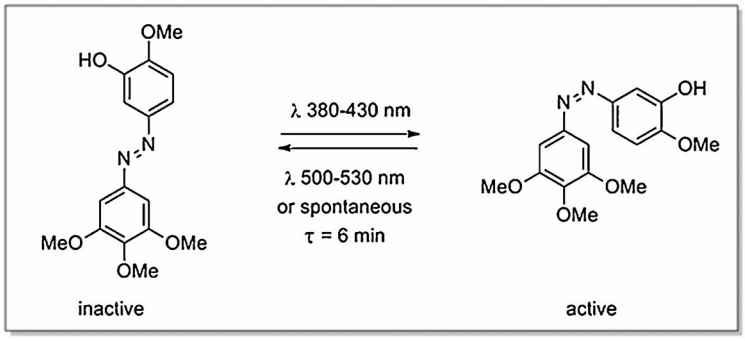
3. Photoswitchable inhibitors of microtubule dynamics

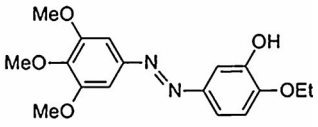
Presently known inhibitors are nonspecific in the sense that their bioactivity can be neither spatially nor temporally directed, e.g. against selected cells and tissues, at defined periods. This restricts their range of application in studies because it prevents their use in spatially or temporally addressing the varied processes dependent on microtubule dynamics. In the treatment of cancer, the time and spatial nonspecificity cause severe systemic side effects such as cardiotoxicity and neurotoxicity [40], which limit the doses at which chemotherapeutics can be applied [41]. Photopharmacology [42] is a novel developing method, not yet at the stage of clinical development. It has a potential of application in medicine because it could lead to the photo controlled, reversible and selective addressing of targets in the human body by responsive small components – drugs (by modifying their

structure upon irradiation with light). Photopharmacological agents are bioactive molecules modified with photoswitches, that is, scaffolds that change (Fig. 7).

Photoswitchable inhibitors of microtubule polymerization have recently been synthesized and studied [43–46]. An interesting novel investigation of spatial and time-controlled compounds was presented by Borowiak et al. [43] They designed and synthesized the photostatins (**22**, Fig. 8) as reversibly photoswitchable analogs of combretastatin A-4 (CA-4). Because active cobretastatins have the *Z* -(cis) configuration and *E* -(trans) CA-4 is several orders of magnitude less potent than the *Z* isomer [47,48], they replaced the bridging C¯–C double bond of the stilbenoid combretastatins with an isosteric N¯–N double bond to give the azobenzenes (**22**).

The compound (**22**) can be trans-/cis photoisomerized with full reversibility and excellent photostability over many cycles by low- intensity visible light. The switching of the (**22**) was demonstrated by UV–vis spectroscopy: 380–420 nm light results in approximately 90 % *Z* isomer. Longer wavelengths cause decreasing *Z* percentages until 500–530 nm gives approximately 85 % *E* isomer. Spontaneous (in the dark) isomerization leads to 100 % *E* isomer, with half-lives from 0.8 to 120 min for different compounds (**22**). Next, the authors studied influences of photoswitchable compounds on microtubule. Obtained molecules modulate microtubule dynamics with subsecond response time and control mitosis in living organisms with single-cell spatial precision. In conclusion, the compounds (**22**) are up to 250 times more cytotoxic when lit with blue light than when kept in the dark in applications in cell culture and are promising as a new class of precision chemotherapeutics whose toxicity may be spatiotemporally constrained using light. In other studies, authors [44] reported azo analog of combretastatin CA4 (**22**) and included data for *in vitro* and HeLa MTT assays, which showed increased cytotoxicity of (**22**), like the cytotoxicity observed for pure combretastatin-A4, in the presence of light. In turn, authors [45] discovered that Z- (**22**) azo compound was with ease reduced with glutathione, which can be a potent problem for biomedical applications. However, the biological results of the cytotoxicity of (**22**) in human umbilical vein endothelial cells and adenocarcinoma epithelial cells fit with other reported findings [43,44]. Rastogi et al. [46] have presented a study about combretastatins analogs in which an azo group was incorporated into a molecule containing the key pharmacophore of colchicine. They have performed cell assays on obtained new compounds (Fig. 8) structurally related to the previously reported azo-analog of combretastatin [43–46]. One of these compounds (**23**) showed enhanced potency against HeLa (IC_{50} = 0.11 μM) and H157 cells (IC_{50} = 0.20 μM) for cell studies under 400 nm irradiation and the highest photoactivity (ratio: IC_{50} with irradiation / IC_{50} in dark = 550).





22 **23**

Fig. 8. The structure of analogs of combretastatin A-4 (**22**) and (**23**) (colchicine binding site).

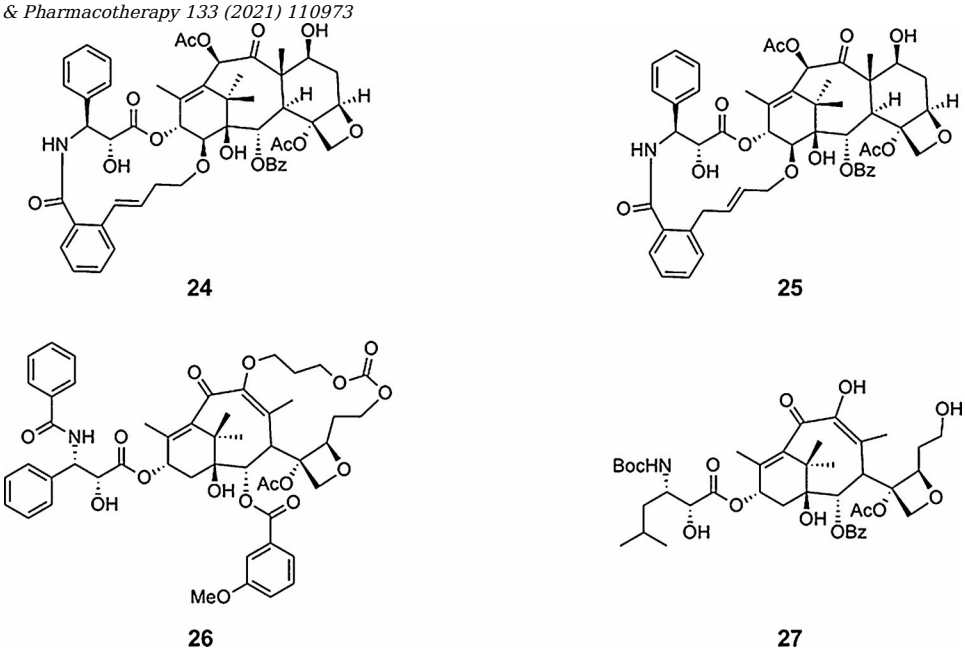


Fig. 9. Novel macrocyclic paclitaxel derivatives (**24**), (**25**) and novel 7,9-O-linked macrocyclic taxoids (**26**) and mimetic of IDN5390 (**27**).

For this compound, authors performed physicochemical and docking studies to investigate ligand binding simulations for the tubulin-combretastatin complex. Kinetic investigations in water reveal a longer half-life for the *Z* isomer of (**23**) which may be the factor responsible for the better IC_{50} values in cell assays and the improved photoresponsive behavior. Basing on docking, it was calculated that the azo compound (**23**) has nearly identical binding conformations and closely overlaps the X-ray binding structure of combretastatin. One difference between combrestastatin A4 and azo (**23**) is the orientation of the para-methoxy group on the A-ring. The calculated binding affinity obtained for (**23**) was -7.6 kcal/mol and was similar to combretastatin and colchicine [46]. The docking calculation was performed for other, less active compounds and binding energies do not indicate a significant difference. In conclusion, compounds (**22**), (**23**) and synthesis of the azo-CA4 analogs represent a promising strategy for switchable potency for the treatment of cancer. However, it should be emphasized that although this novel developing method is attractive, there) are still several limitations with the current photoactivatable MT targeting drugs. The main problem is that the lower energy photons from the UV/Vis range are prone to both scattering in tissue and absorption by endogenous chromophores or they contribute to the photodamage of cells. Feringa et al. [42] presented in recent excellent critical review of the different pharmacological targets (i.e. diseases/organs) in the human body, the prospects for delivering light to these organs, their specific requirements for the molecular design of a photoactivatable drug, and whether and how these challenges can be addressed.

4. Macrocyclic compounds as tubulin inhibitors

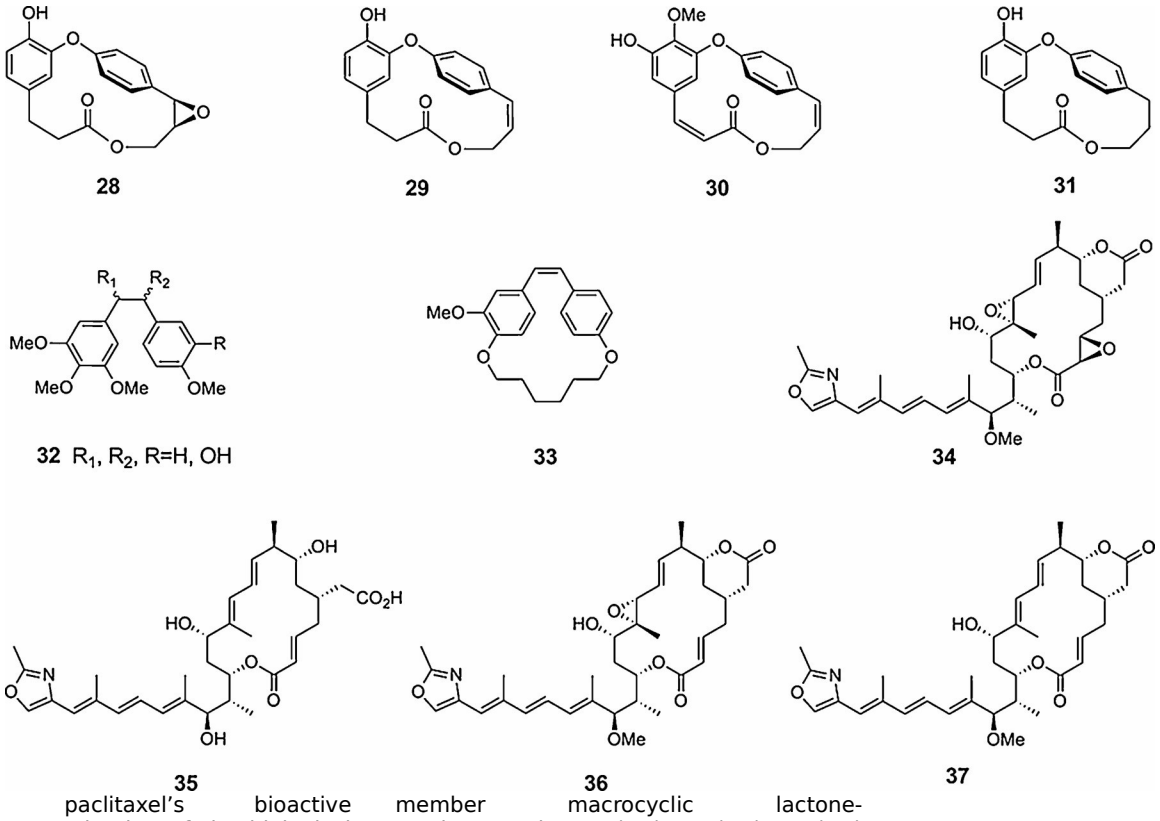
In this part, we will concentrate on macrocyclic compounds that are also tubulin-binding agents. Paclitaxel (**4**) [16] is a macrocyclic compound and chemotherapy medication used to treat various kinds of types of cancer. Novel macrocyclic paclitaxel derivatives were designed to imitate the bioactive conformation of paclitaxel (**24**, **25**) [49] (Fig. 9). Studies revealed that (**24**) induced tubulin polymerization in the absence of G.

TP in a similar way to that of paclitaxel. The microtubules formed with (**24**) as well as paclitaxel were stable against Ca2+-induced depolymerization. The compound (**25**) also exhibited the same or slightly better activity than that of paclitaxel in the same assay (Table 1).

Table 1
Cytotoxicity of the (**24**), of the (**25**) and of the paclitaxel (**4**) against the five cancer cell lines.

Compounds	Five Cancer line - IC50 (nM)				
	ovarian cancer	colon carcinoma	breast cancer		
	A2780	HT-29	MCF-7	NCI/ADR-LCC6-WT	
(24)	183	31	3.49	2.09	17
(25)	592	114	12.3	12.2	29.2
(4)	395	36.1	1.85	2.45	7.28

This result also supports the authors' observation that (**24**) and (**25**) are closely



mimicking paclitaxel's bioactive conformation. Evaluation of the biological activities of (**24**, **25**) novel macrocyclic taxoids has revealed that their potency is highly sensitive to the small conformational differences as well as the rigidity of the compounds. Interesting compounds of novel 7,9-O-linked macrocyclic taxoids (e.g. **26**), mimetic of IDN5390 (**27**) (C-seco taxane more active than paclitaxel in several paclitaxel-resistant human ovarian adenocarcinoma cell lines e.g., A2780TC1, A2780TC3, and OVCAR3, expressing high βIII-tubulin and P-gp levels) [50], together with modification at the C2 position were synthesized. Moreover, their cytotoxicities against drug-sensitive, P-glycoprotein and βIII-tubulin overexpressed drug-resistant cancer cell lines, were evaluated. This study demonstrated that C-seco taxoids showed increased cytotoxicity on drug-resistant tumors overexpressing both III βIII-tubulin and P-glycoprotein. In 1988 seventeen-member macrocyclic lactone-combretastatins D1 (**28**), D2 (**29**), D3 (**30**) and D4 (**31**) (Fig. 10) were discovered [51,52]. Cytotoxicity study (using mouse leukemia cells) which has been reported, showed modest activity (ED_{50} 3–5 μg ml−1) for these compounds [53].

Contrary to the cancer cell growth inhibitory activity exhibited by combretastatin D1, D2, relatively minor structural modifications also caused the elimination of those properties. These groups of cyclic combretastatins, although intensively studied by the group of Pettit in series of "Antineoplastic Agents" [50–53] are less potent as tubulin polymerization inhibitors than the parent CA-4. Another new family of macrocyclic derivatives of combretastatins B (**32**) (Fig. 10) connecting oxygenated substituents on the phenyl rings and indole rings is described [54]. The tubulin polymerization activity for this new class of

Fig. 10. The structure of combretastatins D1 (**28**), D2 (**29**), D3 (**30**), D4 (**31**), the derivative of combretastatins B (**32**), (**33**), and of rhizoxin (**34**) and its analogs (**35**), (**36**) and (**37**) (destabilizing binders- compounds bonding to sites of colchicine).

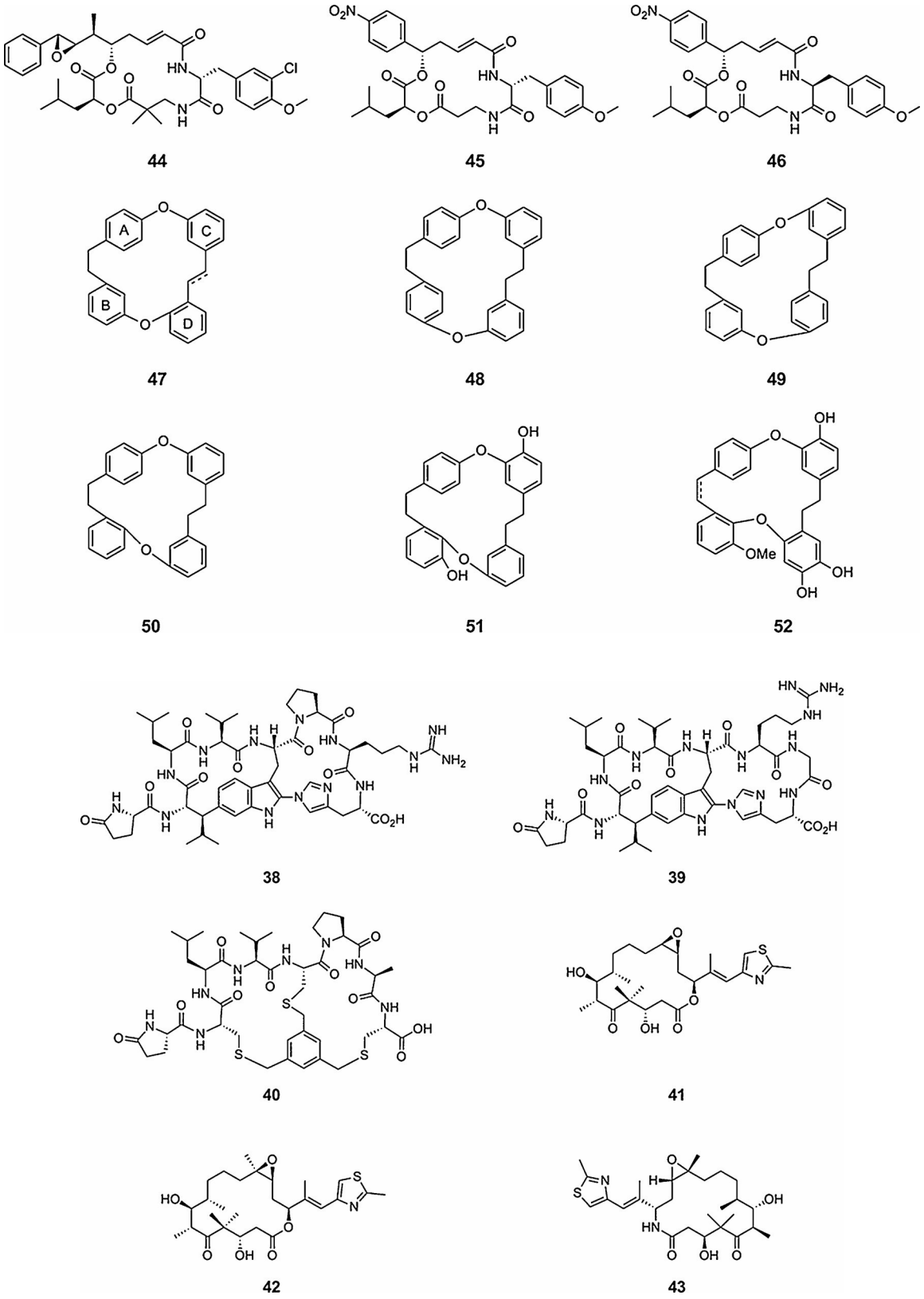


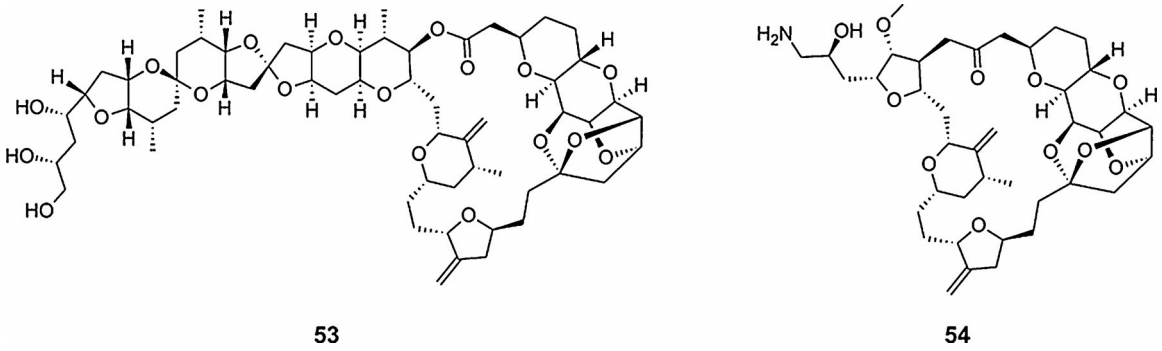
Fig. 11. The celogentin/moroidin family: (**38**), **(39**), (**40**) (compounds bonding probably to colchicine binding site) and the structure of epothilone A (**41**), epothilone B (**42**), and ixabepilone (**43**) (microtubule-stabilizing agents, compounds bonding to site of taxane).

Fig. 12. The structure of (+)-Cryptophycin 52 (**44**), of its derivatives (**45**), (**46**) (microtubule-destabilizing agents, binding site of the *Vinca* alkaloids) and of macrocyclic bisbibenzyls: isomarchantins

(**47**), neomarchantins (**48**), riccardin II (**49**), marchantins (**50**), marchantin C (**51**) and of (**52**) analog (inhibiting
microtubule polymerization).

macrocycles (so-called *p,p*-dihydroxydihydrostilbenophanes) with significant differences in the structural requirements, and the impact of their conformational equilibria on the activity were analyzed. The compound (**33**) was most active in this group in tubulin polymerization inhibitory (TPI) (compound (**33**) 20 μM for 48 % TPI compared to the most active of combretastanine CA4 3 μM for 50 % TPI). The next studied compound was Rhizoxin (**34**) (Fig. 10), which is an antimitotic agent with *anti*-tumor activity [55]. Rhizoxin binds beta-tubulin in eukaryotic cells disrupting microtubule formation [56]. This, in turn, prevents the formation of the mitotic spindle inhibiting cell division. Rhizoxin binds to tubulin in the maytansine site and depolymerizes assembled microtubules [57]. Derivative compounds of rhizoxin (**35**), (**36**) and (**37**) (Fig. 10) have improved biological activity [58].

The celogentin/moroidin family (**38**, **39**) (Fig. 11) of cyclic peptides was isolated from the seeds of the cockscomb plant, *Celosia argentea*, and exhibited potent inhibition of tubulin polymerization [59]. The most potent member of the family, celogentin C (**38**), presented greater inhibitory activity than vinblastine [60]. Another compound - the moroidin (**39**), also inhibited tubulin polymerization and is a peptide composed of eight amino acids, with unusual leucine-tryptophan and tryptophan-histidine cross-links that form its two rings. Celogentin C (in the same family of compounds) is the most potent (IC_{50} 0.8 μM) - even more potent than the *anti*-mitotic agent vinblastine (IC_{50} 3.0 μM) [56]. Moroidin has the same potency as vinblastine [61]. Because of its biological activity, compounds in this family are still being tested as *anti*-cancer agents (compound **40**, IC_{50} = 2.2 μM [62]. The following microtubule-stabilizing macrolides, which were first isolated from the myxobacterium *Sorangium cellulosum* by Reichenbach, Hofle, et al. [¨ 63] in 1987, are epothilones A (**41**) and B (**42**) (Fig. 11). Epothilones have served as successful lead structures for the development of new anticancer drugs [64]. The inhibition of cancer cell growth by epothilones is based on the suppression of cellular microtubule dynamics, which leads to cell-cycle arrest in G2/M and the induction of apoptosis [65]. Epothilones are interacting with β-tubulin in the taxol/epothilone binding cavity [66]. Epothilone B (**42**) and several of its analogs have been advanced into clinical trials on humans, and one of these compounds, ixabepilone (**43**) also known as BMS-247550, was approved by the US Food and Drug Administration for breast cancer treatment in 2007, under the name Ixempra [67]. It should also be emphasized that the structure-activity relationships (SAR) of epothilones have been studied extensively, including the effects of changes in the configurations of the various chiral centers, which is well described by the group of Altmann [68]. The Cryptophycins are members of the macrocyclic depsipeptide family produced by cyanobacteria of the genus Nostoc [69]. This group of compounds inhibits the polymerization of tubulin by blocking microtubule growth, destabilizing them in *Vinca* building domain. They are the most potent compounds known in the dynamics of tubulin and display remarkable cytotoxicity against multidrug-resistant cancer cell lines [70].



53 54

Fig. 13. Halichondrin B (**53**) and Eribulin (**54**) microtubule destabilizing agents, compounds that bind to the *Vinca alkaloid* site.

In recent years extensive research has been done on structural modifications of these molecules [71]. They led to the synthesis of (+)-Cryptophycin 52 (LY355703) (**44**) (Fig. 12), which reached phase 2 clinical trials [72]. The multinational pharmaceutical company Sanofie-Aventis also noticed the potential in this group of compounds and published two patents on cryptophycin antibody-drug conjugates (ADCs) for the treatment of lung cancer [73]. Studies on synthesized analogs of cryptophycin are still underway. Shah et al. [74] designed and synthesized novel cryptophycins based macrocyclic depsipeptides with a truncated epoxide chain (**45**) and (**46**) (Fig. 12). The compounds (**45**) and (**46**) exhibited good *anti*-cancer activity, induced apoptosis,

blocked/delayed G2/M cell cycle as well as significantly reduced the expression of α- and β-tubulins. The next series of compounds in which tubulin inhibitors are sought for is macrocyclic bisbibenzyls [75]. This is the group of phenolic natural products that are mainly found in bryophytes [76]. The bisbibenzyl skeleton comprises four aromatic rings (arene A–D) and two ethano bridges (**47**), (**48**), (**49**) and (**50**) (Fig. 12). Isomarchantins (**47**), neomarchantins (**48**), riccardin II (**49**) and marchantins (**50**) are four main sub-classes of macrocyclic bisbibenzyls that possess two diaryl ether sub-units as a common structural feature. In turn, marchantin C (**51**) (Fig. 12), a natural macrocyclic bisbibenzyl, was discovered to be a potent tubulin inhibitor that targets the colchicine-binding site.

This compound was also found to exhibit multidrug resistance- reversing activity by inhibiting the P-glycoprotein [77]. Because its bisbibenzyl scaffold is similar to the cis-stilbenoid *anti*-tubulin agents, such as combretastatins (eg. **10**, **11**), a series of macrocyclic bisbibenzyls with novel skeletons was designed, synthesized and evaluated for antiproliferative activity against five anthropic cancer cell lines [77]. It was discovered that molecule (**52**) (Fig. 12) showed excellent anticancer activity against cervical cancer cells, chronic myelogenous leukemia, breast cancer cells, Caucasian colon adenocarcinoma grade II and prostate cancer cell line, with IC_{50} values ranging from of 1.51 μM–5.51 μM. Compound (**52**) also effectively inhibited tubulin polymerization in HCC1482 cells and induced HCC1482 cell cycle arrest at the G2/M phase, at the colchicine-binding site. Next important macrocycle is halichondrin B (**53**) (Fig. 13) isolated from sea sponges of the genus *Halichondria and Axinella* [78]. Lack of universal availability of a natural compound has necessitated on scientists the creation of artificial halichondrin and its analogs. Retaining similar properties to the natural product, chemical compounds are classified as non-competitive *vinca* alkaloid inhibitors, because their microtubule binding site is not identical to the above domain. An example of a simple analog halichondrin B is compound Eribulin ((**53**), E7389, Fig. 13), which was approved by the U.S. Food and Drug Administration on November 15, 2010 to treat patients with metastatic breast cancer who have received at least two prior chemotherapy regimens for late-stage disease, including both anthracycline- and taxane-based chemotherapies [79]. There was an interesting study of this compound in 2016. Doodhi et al. [80] demonstrated by X-ray microscopy that the binding of a single eribulin molecules to a growing microtubule end could cause MT lattice defects or induce MT catastrophe (depolymerization). The authors propound that eribulin amplifies a natural pathway toward catastrophe by promoting the arrest of protofilament elongation. The researchers proposed a general strategy to investigate and manipulate the dynamics of cytoskeletal arrays at high resolution.

In conclusion, microtubule drugs have been vital to advances both in medicine and basic sciences. Presented in this work derivative of stilbene, in particular, combretastatins, have taken the interest of the research community since the discovery of their remarkable biological activities. Such compounds as combretastatins analogs combretastatin A-1(CA1, **7**) combretastatin A-4 (**8**) and their three prodrugs of monosodium phosphate salt two CA-1 P (Oxi4503, **9**) CA4P (Fosbretabulin, **10**) amino derivative AVE8062 (Ombrabulin, **11**) along with biologically (**12-15**) and dibenzo[b,f]oxepin derivatives have been extensively studied and some are already in use. Macromolecular conjugates and nanocarriers of combretastatin CA4, have been developed and were successful in improving drug solubility and bioavailability. Polymer- based systems of CA4 (PLG-CA4, **17**) were able to persistently disrupt the vasculature of a murine colon C26 tumor model with a tumor suppression rate of 74 % as opposed to 24 % obtained with CA4P. We presented in this work both of the already-introduced drugs e.g. paclitaxel (4) as well as potential drugs and research directions that are being conducted to obtain new drugs. The direction of creating macrocyclic compounds seems to be very attractive, as well as the newly discovered method of photopharmacology.

Declaration of Competing Interest

The authors report no declarations of interest.

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